Abstract

Rapid urbanization has led to the generation of enormous wastewater after independence. The domestic wastewater generated in municipalities is rich in nutrients such as carbon, nitrogen and phosphorus along with other ions. The generated wastewater due to lack of adequate appropriate infrastructure including low treatment efficiencies are either untreated or partially treated and are let into water bodies. Present sewage treatment plants (STP's) in the city are either under capacity or malfunctioning and hence are unable to meet the growing demand of burgeoning urban population. Water bodies have the ability to uptake nutrients (remediation by algae, bacteria, macrophytes) provided the wastewater inflow does not exceed the threshold. However, the sustained flow of wastewater beyond the water body's treatment ability has led to the serious problem of nutrient enrichment in surface water bodies which is evident from algal bloom and profuse growth of invasive exotic macrophytes. This necessitates cost effective environmentally sound treatment options. The current research focuses on the characterisation of domestic wastewater fed ponds/lakes, understanding of nutrient regimes in wastewaters, pond dynamics, nutrient transformation and resource recovery. This has aided in devising an algae based treatment system for Bangalore city.

The interplay between various biotic and abiotic factors governs water quality in a water body. Regular monitoring helps in characterisation of the water body and also helps in identifying the sources of external input (if any) to the system. Wastewater generated in urban localities in India, due to lack of adequate appropriate infrastructure including low treatment efficiencies are untreated or partially treated and are let into water bodies. Understanding the nature of the wastewater flow regimes and the turnover of biota with prevalent nutrient conditions is required to design treatment systems. Treatment involves breakdown of complex organism forms into simpler forms and transformations of organic nutrients into inorganic forms that are finally absorbed and assimilated by microbes as algae and bacteria. In wastewater fed urban pond systems, an array of microphytes as well as macrophytes grow and help in nutrient cycling in the system and still manage to remove nutrients to satisfactory levels. However, sustained inflow of wastewater with high nutrients results in the deterioration of the system as nutrient input exceed the supportive and assimilative capability resulting in proliferation of macrophytes, algal blooms, froth formations rendering the system anoxic that results in the loss of functional abilities of the urban pond systems. This biota in the system plays a major role in nutrient removal and recycles. Understanding the nutrient cycling aspects of urban wastewater fed systems is essential to find out the key players in treatment and for devising a sustainable treatment option with resource recovery.

The review of wastewater generation, treatment systems highlight shortfall of the treatment systems and need for sustainable treatment for removal and recovery of nutrients such as C, N and P. Characterisation of Varthur water body (spatial extent 220 ha) located in the south of Bangalore city has been done through monthly monitoring for

18 months with the analyses of physico-chemical and biological. The analysis showed BOD removal of 70% (filterable) when the lake functioned as an anaerobic–aerobic lagoon for 6 months at an estimated residence time of 5 days. During this period, the biota of the lake, especially primary producers such as algae, treat the water through remediation of nutrients to nearly standard water quality levels. However, the growth and spread of invasive exotic macrophytes such as water hyacinth rendered the lake anaerobic which reduces its ability (due to absence of low algae) to treat the water. This highlights the role of algae especially Chlorophycean members as *Chlorococcum* sp., *Chlorella* sp. and *Monoraphidium* sp. in treating urban domestic wastewater and the scope for introducing algal ponds/lagoons to treat wastewater treatment and it may be used in a larger number of small towns to enable local reuse of water.

The entire pond systems comprises of various components that are deeply affected by the biotic and abiotic factors in the system. Hence, studies on major biotic components were conducted especially on algae and macrophytes and the impact of abiotic factors as wind, light, and precipitation with seasonality's. The diurnal and spatio-temporal variations in the dissolved oxygen as well other treatment parameters were used for zonation through multivariate analysis. Physico-chemical parameters confirm the nutrient enrichment (high Amm.-N) in the water body due to the sustained inflow of wastewater. High levels of nutrients together with BOD have resulted in the lower DO levels affecting the biological life. Study on biota revealed macrophytes altering the photosynthetic regime in the algae in water bodies thus, creating anoxia and nutrient re-suspension. The multivariate analysis showed three distinct zones (clusters) on the basis of physico-chemical variables and nutrient concentrations in the lake. The sedimentary C and N analysis showed a steady increase in the C: N ratio as a function of residence time.

Importance of the various sub-systems in the water body in terms of nutrient uptake and accumulation showed algal systems to be efficient. C budgeting accounted to ~ 7 t/d i.e. ~2574 t/y, indicated that the lake is an accumulator of C. An estimated relatively high gas emission across the water/air interface (17 t/d) to carbon burial into sediments (2.3 t/d) further indicates very high emissions compared to sedimentation showing the dominance of internal C cycles. The overall mass balance, gas exchange and carbon burial balance showed Varthur water body as a major emitter of C due to high primary production, substantive allochthonous carbon inputs and intensive anthropogenic activities in the water body. Gaseous carbon emission accounted for 28 % of the total Influx C. The spatial profile of N in sediment ranged from 2280-3539 mg/kg of sediment dry mass. Very low value of N:P ratio in sediments suggested possible N limitation. The determination of ammonification and nitrification showed lower nitrification rates than the ammonification rates. The potentially mineralisable nitrogen content in Varthur pond sediments varied greatly from 21.65% to 75.54% and was strongly correlated (r^2=0.85) to sedimentary TN. N budgeting showed NH_4-N as the predominant N_r form for microbial uptake and is the major mechanism for nitrogen removal, followed by the sedimentation process. Bacterial biomass, algal biomass and macrophyte biomass accounted for 14, 4

and 1% N removal, respectively. Ammonium concentration and nitrification accounted for 27% and 2%, respectively. While bacterial uptake remained fairly constant throughout the year, micro-algae was the major player during monsoon and winter and macrophytes dominated N_r capture during summer among autotrophs. From the estimates, it has been observed that nearly 55% N_r was recovered, recycled as cell mass and transferred to a crop system when such N-captured water is used for irrigating fodder crops. About 45% of N input into the system was lost and methods to reduce this loss need to be evolved in the future. The spatial profile of P in sediment ranged from 2111.35-3982.03 mg/kg of sediment dry mass. Inorganic-P (IP) ranging from 1270.27-3505.73 mg/kg was found to be the major fraction (61.16-91.56%) of sedimentary P. High p values in both water columns and sediments showed potential P excess conditions. P concentrations in micro and macro-algae collected during the due course of the study (on dry biomass basis) were 0.347% and 0.939% P respectively.

The P fractionation revealed metal oxide bound P (NaOH-P) and constituted major fraction of IP indicating, high concentrations of Fe and Al in sediments. High concentrations of sedimentary N and P indicated possible higher trophic status (bio-productivity/unit volume) signifying its towering nutrient status evidenced from the rank order of P fractions: NaOH-P > HCl-P > NH_4Cl-P, which is specific for highly enriched water bodies. P budgeting showed that bulk of the P is trapped in sediment layer with a potential of ~50 % recovery from the sediments indicating, ~70% P retention within the system. The biotic components such as bacteria, algae and macrophytes accumulates a substantial amount of P, immobilising ~139, ~482 and ~131 tonness/yr of P. The sequential P extraction shows that ~70 % of sediment bound P is readily reducible during anoxic conditions which can potentially become bio-available to trigger algal growth.

Assessment of treatment efficiency of facultative algal ponds, showed moderate treatment levels with 60 % total COD removal, 50% of filterable COD removal; 82% of total BOD removal and 70% of filterable BOD removal. The N removal efficiency was lower. However, a rapid decrease in the suspended solids after a faster euglenoids growth indicated particulate C removal by algal ingestion. Euglenoides dominated the facultative pond and Chlorophycean members were more abundant in the maturation ponds owing to variable surface BOD loadings. Significant correlations between algal biomass and nutrients indicate the importance of the type and nature of algal communities that can be used as an efficient tool for predicting the dynamics of various phases in wastewater treatment systems.

Detailed morphological analysis of dominant algal species i.e. euglenoides was also performed. Euglenophycean members (>14 species) sampled from various locations in the facultative pond based system showed various striae patterns and distinct nano channels on the cell surface that might have possible role in cell secretions. Comparative assessment of treatment systems reveal that algal pond systems performed well under higher organic load with a COD removal efficiency of 70%, TN removal efficiency of

73% and TP removal efficiency of 22%. However, the facultative pond based systems were effective in suspended solid (SS) removal up to 93% and BOD removal up to 82%. The conventional wastewater treatment systems were efficient in terms of SS removal up to 88%, COD removal up to 74% and BOD removal up to 63%, but were highly ineffective in nutrient removal.

The evaluation of treatment processes in mechanically aerated systems, facultative ponds and large shallow lake based systems in terms of capital and annual O&M costs, COD removal cost and land requirements reveals that the mechanical systems require 5 times more capital and O&M costs than ponds. The treatment systems were also ranked in terms of the total annual cost (e.g., capital, manpower, chemical, repair, electricity, land). It showed that algal pond systems followed by facultative pond based system are economically better choice than mechanical technologies. Finally, it was found that the large pond based systems could be economically the best option for the developing countries considering all factors, including economic viability and treatment efficiency. The treatment efficiency analysis showed that algal pond systems were the most effective options for treating urban wastewater.

Culturing native wastewater species in growth media and wastewaters, assessment of efficient cell disruption and solvent systems, lipid profiles of wastewater algal species were studied. Wastewater grown algal species as *Euglena* sp., *Spirogyra* sp., *Phormidium* sp., *Lepocinclis ovum*, and *Chlorococcum* sp. are comparatively rich in lipids. These algae grow mixotrophically and can store substantial amount of wastewater carbon as TAG's in varied environments. Among the different cell disruption methods used for the study, sonication was the most effective. The combination of maceration and methanol: chloroform: water (2:1:0.8, v/v) (Bligh and Dyer's, solvent) gave highest lipid extraction yield among other combinations. Further more these wastewater algae as *Lepocinclis ovum* and *Chlorococcum* sp. were found to grow better in wastewaters. Increased lipid content was recorded during the cell cultures with accumulation of quality FAME with high saturates predominated by C16-C18 fatty acids. These wastewater algal lipids are suitable for bio-energy generation with potential biomass productivity (6.52 t/ha/yr) of wastewater-grown species as *Euglena.*

The studies on *Euglena* sp. showed mixotrophic mode that offers an efficient removal of TOC, N and P from domestic wastewater without any pre-treatment. Lipid profiles of the extracted algal oil were similar to the vegetative feedstock oils, indicating a good quality fuel for energy generation. Mixed algal consortia's bioremediation potential (removal of nutrients) with the scope for biofuel production highlights self flocculating abilities of algal consortia aided in the effective treatment of wastewater with substantial algal harvest.

Studies on cultivating wastewater algal consortia in novel cascading algal parcel flow reactor (CAPFR) operating in continuous mode showed 70-80 % nutrient and ~90 % C removal with in a residence time of ~4 days with highest cell densities (0.91 g/l) and

productivities (0.26 g/l/d) in the last stages. The lipid contents varied from 26-28 % with highest lipid productivities ~58 mg/l/d in the 2nd phase of the bioreactor. Most of the lipids were associated with the pigments as chlorophyll and carotenoids. Furthermore, the algal rector removed bacteria up to 4 log orders. Essential cations and phosphates were responsible for self clumping of algal biomass in the final stages with a high internal P content within the cell. The algal biomass also showed substantial exothermic peaks and high heat values (~18 MJ/kg). Studies on continuous cultivation of *Dictyosphaerium* sp. showed that this species could adapt to wastewater conditions and also showed good nutrient removal at lower HRT (2.5 days). The high biomass productivities with high lipid content (~36%) at low HRT in the continuous mode offer potential options for economic and feasible nutrient removal with biofuel production.

Investigations on city wastewaters showed low nutrient ratio indicating C limitations and possible scope for algal wastewater treatment. Integration of algal ponds in the present treatment network requires an additional land ranging from ~0.37 to 2.75 ha to treat an MLD of wastewater depending on the nutrient content and influent waters fed to algal systems. The treatment plants require an additional ~1.6 ha of land to treat 1 MLD of wastewater considering an average N and P values of 18 and 5 mg/l. The continuous algal bio-processes implemented at decentralised levels would help in the economical ways for nutrient removal and recycling of the nutrient free waters after treatment. This meets multiple objectives of low cost treatment of wastewater, nutrient recovery and fuel production. Algal nutrient capture and consequent biofuel production would ensure sustainability through i) water purification ii) nutrients capture and iii) biofuel to meet the growing energy demand, and would be an optimal treatment option for urban wastewater. The thesis consists of 10 chapters and basically deals with the development of a sustainable and economically viable bioprocess for wastewater treatment and biomass production.

Chapter 1 provides a brief introduction to wastewater; domestic wastewater composition, generation and treatment in developing nations and in the country and review of the various techniques for treatment of domestic wastewaters, advantages of algal processes in nutrient removal (C, N and P) and production of valued by-product such as lipid generation, for its use as biofuel.

Chapter 2 is based on primary field investigations in a wastewater fed urban water body/pond systems involving monthly sampling and analysis of various physico-chemical and biological parameters. Assessment of treatment capabilities of the continuous systems through detailed characterisation of treatment parameters is explained in the second chapter.

Chapter 3 discusses the role of the major biotic (algae and macrophytes) and abiotic factors in nutrient transformations, the diurnal variations in parameters especially dissolved oxygen, multivariate spatio-temporal analysis of functional abilities for zoning,

the activities in the sludge/sediment and transitions in the CN ratio as a function of residence time.

Chapter 4 involves studies in C, N and P quantification and budgeting in such pond systems and partitioning of the nutrients and their distribution in various biotic and abiotic subsystems. This chapter also highlights the major nutrient losses from the system and un-utilised nutrient stocks, paving way for beneficial use of nutrients from such man made lagoon wastewater systems.

Chapter 5 discusses the mechanisms and efficacies of algal pond based treatment systems through a detailed study and highlight its advantages over the mechanical ASP based systems. This has been done through a comparative assessment of treatment efficiency, economics and environmental externalities. This study also provides necessary insights and potential of wastewater algal species such as *Euglena* for its abilities in nutrient removal and biomass generation. This provides insights to algal treatment options for optimal resource recovery and utilisation from wastewaters.

Chapter 6 focuses on testing the growth, biomass and lipid production of various wastewater algae isolated from treatment ponds. The chapter identifies suitable cell disruption and extraction routes for efficient lipid extraction and assesses the potential of these wastewater grown algae for regional and national biofuel production.

Chapter 7 discusses the effectiveness of wastewater grown *Euglena* sp. and algal consortia in nutrient removal and as a source of lipids for biofuel generation.

Chapter 8 involves the design and operation of a continuous algal (uni-algal/algal consortia) bioreactor devised taking insights from earlier field based studies and their potential as efficient urban wastewater treatment systems.

Chapter 9 discusses the present nutrient levels in the city wastewaters and also an analysis of the temporal and spatial variation of nutrients in city sewers and elaborates the scope for integration of the algal modules i.e. continuous algal bioreactors (designed in the previous chapter) into existing STP’s.

Chapter 10 elaborates significant contributions and outcome of the research.